Food Chemistry 206 (2016) 234–238

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short communication

Pinoresinol of olive oil decreases vitamin D intestinal absorption

Aurélie Goncalves [a,b,c], Marielle Margier [a,b,c], Camille Tagliaferri [d], Patrice Lebecque [d], Stéphane Georgé [e], Yohann Wittrant [d], Véronique Coxam [d], Marie-Josèphe Amiot [a,b,c], Emmanuelle Reboul [a,b,c,*]

[a] INRA, UMR1260 "Nutrition, Obesity and Risk of Thrombosis", F-13385 Marseille, France
[b] INSERM, UMR U1062, F-13385 Marseille, France
[c] Aix-Marseille Université, F-13385 Marseille, France
[d] INRA, UMR 1019, UNH, CRNH Auvergne, F-63000 Clermont-Ferrand, France
[e] Biochemistry Department, Centre Technique de Conservation des Produits Agricoles (CTCPA), Site Agroparc, F-84911 Avignon, France

ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form 2 March 2016
Accepted 15 March 2016
Available online 16 March 2016

Keywords:
Cholecalciferol
Uptake
Polyphenol
Enterocyte
Caco-2 cells
Rats

ABSTRACT

Enriching oils, such as olive oil, could be one solution to tackle the worldwide epidemic of vitamin D deficiency and to better fit with omega 3 (DHA) recommendations. However, data regarding the interactions occurring at the intestinal level between vitamin D and phenols from olive oil are scarce. We first determined the effect of polyphenols from a virgin olive oil, and a virgin olive oil enriched with DHA, on vitamin D absorption in rats. We then investigated the effects of 3 main olive oil phenols (oleuropein, hydroxytyrosol and pinoresinol) on vitamin D uptake by Caco-2 cells. The presence of polyphenols in the olive oil supplemented with DHA inhibited vitamin D postprandial response in rats (−25%, $p < 0.05$). Similar results were obtained with a mix of the 3 polyphenols delivered to Caco-2 cells. However, this inhibitory effect was due to the presence of pinoresinol only. As the pinoresinol content can highly vary between olive oils, the present results should be taken into account to formulate an appropriate oil product enriched in vitamin D.

1. Introduction

An adequate vitamin D status appears highly beneficial for human health to prevent bone diseases (Holick, 2007), sarcopenia (Lappe & Binkley, 2015) and even some cancers (Cannell, Hollis, Zasloff, & Heaney, 2008; Lappe, Travers-Gustafson, Davies, Recker, & Heaney, 2007). As sun exposure is not sufficient to reach an adequate vitamin D status in most people, vitamin D should be provided in sufficient amounts by the diet (Holick & Chen, 2008), i.e. 15 μg *per* day for healthy adults (2010). Unfortunately, this recommendation is particularly difficult to reach (Maillot et al., 2009) and 75% of the US population is vitamin D deficient (Adams & Hewison, 2010) – a report that can be extended worldwide (Mithal et al., 2009). Enriching oil products such as olive oil, which is used as a staple in various diets, would thus be an interesting solution to tackle this worldwide epidemic of vitamin D deficiency.

We previously reported that oleic acid, which represents 80% of the olive oil fatty acid content, is the fatty acid that favors the most vitamin D basolateral secretion by intestinal cells (Goncalves et al., 2013). Olive oil would thus be a good food vector for vitamin D enrichment. However, data reporting interactions occurring at the intestinal level between vitamin D and the other components of olive oil such as phenolic compounds are scarce. Interestingly, vitamin D and α-tocopherol share common uptake pathways (Reboul et al., 2011) and an inhibition of α-tocopherol uptake by Caco-2 cells has already been observed in the presence of naringenin, a polyphenol of grapefruit juice (Reboul et al., 2007b).

We thus first investigated the effect of virgin olive oil compared to refined olive oil (without polyphenols), supplemented, or not supplemented, with DHA, on vitamin D absorption in rats. We then aimed to identify the competitive or synergistic interactions between vitamins D and the 3 main polyphenols from olive oil during their uptake by enterocytes using Caco-2 cells.

2. Materials and methods

2.1. Chemicals

Cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2), 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (phosphatidyl-

Abbreviations: AUC, area under the curve; FBS, fetal bovine serum; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium; SR-BI, Scavenger Receptor class B type I.

* Corresponding author at: UMR 1062 INSERM/1260 INRA/Aix-Marseille Université, Faculté de Médecine la Timone, 27 boulevard Jean-Moulin, 13385 Marseille Cedex 5, France.
E-mail address: Emmanuelle.Reboul@univ-amu.fr (E. Reboul).

http://dx.doi.org/10.1016/j.foodchem.2016.03.048
0308-8146/

choline), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium (MTT), 1-palmitoyl-sn-glycero-3-phosphocholine (lysophosphatidylcholine), monoolein, free cholesterol, oleic acid and sodium taurocholate were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose and trypsin–EDTA (500 mg/L and 200 mg/L, respectively), non-essential amino acids, penicillin/streptomycin and PBS were purchased from Life Technologies (Illkirch, France), and fetal bovine serum (FBS) came from PAA (Vélizy Villacoublay, France). The refined olive oil (without phenols) and the virgin olive oil and were provided by Lesieur Inc. (Asnières-sur-Seine, France). The phenol content of the virgin olive oil is presented in Table 1. Oil was supplemented in DHA using fish oil enriched in DHA (marine fish oil concentrate containing 45% DHA) obtained from Lipid Nutrition (Koog aan de Zaan, the Netherlands).

2.2. Preparation of vitamin D-enriched vehicles for rat and cell experiments

2.2.1. Preparation of vitamin D-rich emulsions

To deliver vitamin D to rats, olive oil-in-water emulsions were prepared as follows. An appropriate volume of cholecalciferol stock solution was transferred to eppendorfs to obtain a final amount of 600 μg in each tube. Stock solution solvent was carefully evaporated under nitrogen. Dried residue was solubilized either in 800 μl olive oil or in 800 μl of a mix of olive oil:fish oil (750:50; v:v) providing 36 mg DHA. Four hundred μl of NaCl 0.9% solution were then added. The mixture was vigorously mixed by sonication (Branson 250 W sonifier, Osi) for 20 s and used for force-feeding within 3 min.

2.2.2. Vitamin D-rich micelles

For delivery of vitamin D to Caco-2 cells, mixed micelles were prepared as previously described (Goncalves et al., 2011, 2013; Reboul et al., 2011). Briefly, appropriate volumes of the following compounds were transferred to glass bottles to obtain the following final concentrations: 0.04 mM phosphatidylcholine, 0.16 mM lysophosphatidylcholine, 0.3 mM monoolein, 0.1 mM free cholesterol, 0.5 mM oleic acid and 0.5 μM cholecalciferol. Stock solution solvents were carefully evaporated under nitrogen. The dried residue was solubilized in DMEM containing 5 mM taurocholate and vigorously mixed by sonication at 25 W (Branson 250 W sonifier; Danbury, CT, U.S.A.) for 3 min. The mixtures obtained were sterilized and filtered by passing them through a presterilized 0.22 μm filter (Millipore). Phenols were added extemporaneously in DMSO at different concentrations depending on the conditions (see Fig. 2). The final DMSO content in all micelle lots was 0.1%. Concentrations of vitamin D in the micellar solutions were checked using HPLC before each experiment.

2.3. Characterization of vitamin D absorption in rats

Thirty-nine-month-old Wistar female rats were purchased from Janvier (Janvier, Le-Genest-St-Isle, France). The rats were housed in a temperature-, humidity- and light-controlled room. They were given a standard chow diet and water *ad libitum*. Rats were fasted overnight before each experiment.

On the day of the experiment, the first kinetic point was performed at fasting (zero baseline sample) by retroorbital puncture under sevoflurane anesthesia. The rats were then force-fed with the vitamin D-enriched olive oil-in-water emulsions and additional blood samples were taken at 1, 2, 4, 6 and 8 h after force-feeding. The last blood sample was harvested by intracardiac puncture under sevoflurane anesthesia before rats were euthanatized. Plasma samples were stored at −80 °C until vitamin D analysis by HPLC. Results were expressed as μmol vitamin D/ L of plasma, or as μmol vitamin D/ gram of total plasma lipids. All experimental procedures were approved by the institution's animal welfare committee (Comité d'Ethique en Matière d'Expérimentation Animale Auvergne: CEMEAA, Agreement No. C6334514) and were conducted in accordance with the European Union's guidelines for the care and use of laboratory animals (2010- 63UE). All efforts were made to minimize animal suffering.

2.4. Cell culture

2.4.1. Caco-2 cell culture

Caco-2 clone TC-7 cells were cultured as previously described (Reboul et al., 2005, 2006). For each experiment, cells were seeded and grown on transwells for 21 days as previously described (Reboul et al., 2005, 2006) to obtain confluent and highly differentiated cell monolayers. Twelve hours prior to each experiment, the media in apical and basolateral chambers were replaced with serum-free complete medium.

2.4.2. Cytotoxicity assay

Polyphenol cytotoxicity in the cell culture medium was assessed using growing concentrations from 50 to 500 μM. Cell viability was determined using an MTT assay. Briefly, Caco-2 cells were seeded and grown for 3 days in 96-well plates, prior to a 1 h-incubation polyphenol treatment. Cell culture medium was then removed, and the MTT solution (1 mg/ml) was added for 2 h at 37 °C. The supernatant was then replaced by DMSO (100 μl per well) and the absorbance was determined at 540 nm.

2.4.3. Characterization of vitamin D apical transport in the presence of polyphenols in cells

Apical uptake of vitamin D incorporated in mixed micelles was determined after a 1 h-incubation as previously described (Goncalves et al., 2011, 2013, 2015; Reboul et al., 2011). Absorbed vitamin D was estimated as vitamin D present in harvested cells. Results were expressed as percentages of vitamin D absorbed compared to the control condition without polyphenols (control set at 100%).

All the samples (harvested cells and culture media after incubation) were sealed under nitrogen and stored at −80 °C until analysis. Aliquots of cell samples were used to assess protein concentrations using a bicinchoninic acid kit (Pierce, Montluçon, France).

2.5. Polyphenol analysis

Extraction of phenolic compounds from oil was carried out using 1 ml of syringic acid (0.015 mg/ml in methanol and used as internal standard) which was added at 2 g of olive oil before being

Table 1
Olive oil phenolic content.

Phenol class	Secoiridoids	Flavonoids	Lignans	Phenylethanoids
Main molecules	Oleuropein derivatives	Luteolin	Pinoresinol	Hydroxytyrosol and tyrosol
μg/100 g	29920.8 ± 255.7	28407 ± 3.0	4990.4 ± 75.7	1903.8 ± 18.0

Data are mean ± SEM, $n = 4$.

evaporated under nitrogen. Then 6 ml of a methanol/water (80/20) mixture was added and vigorously mixed for 5 min. To finish, the extraction mixture was centrifuged at 3500 g during 25 min.

The phenolic compounds were then quantified using an HPLC system (Agilent 1200 series) equipped with a diode array detector. Separation was performed on a column Waters Spherisorb ODS-2 (C18; 5 μm; 4.6 mm × 250 mm). The mobile phase consisted of a gradient of water and 0.2% of phosphoric acid (A), methanol (B) and acetonitrile (C). Flow rate was 1 ml/min. The gradient profile of the mobile phase (A:B:C) was set at 96:2:2 and changed linearly to 50:25:25 for 40 min. It was then changed to 40:30:30 from 40 to 45 min, and to 0:50:50 from 45 to 60 min. This last ratio was maintained during 10 min and the mobile phase was finally changed back to 96:2:2 from 70 to 72 min. The detection was performed at 280 nm and the injection volume was 20 μl. Results were expressed as μg phenolic compound/ 100 g oil.

2.6. Vitamin D analysis

Vitamin D was extracted from 500 μl aqueous samples using the method previously described (Goncalves et al., 2011, 2013; Reboul et al., 2011). The internal standard was ergocalciferol (vitamin D_2). After lipid extraction with hexane, dried residues were dissolved in 200 μl of mobile phase (60% acetonitrile – 38% methanol – 2% water). A volume of 180 μl was used for HPLC analysis.

The HPLC system comprised a Shimadzu separation module (LC-20ADSP HPLC Pumps and SIL-20CHT Autosampler; Shimadzu, Champs-sur-Marne, France), and a SPD- M20A Shimadzu photodiode array detector (detection at 265 nm, spectral analysis between 190 and 300 nm). Cholecalciferol and ergocalciferol were separated using a 250 × 4.6 mm RP C18, 5-μm Zorbax column (Interchim, Montluçon, France) and a guard column. Quantification was performed using Chromeleon software (version 6.50 SP4 Build 1000, Dionex) comparing peak area with standard reference curves.

2.7. Plasma lipid analysis

Three aliquots (5 μl) of each plasma sample were used for total cholesterol, phospholipid, and triacylglycerol assays with kits from Biolabo (Maisy, France) according to the manufacturer's instructions. For lipids standardization, plasma vitamin D amounts were corrected for total lipids (total cholesterol + phospholipids + triacylglycerols).

2.8. Statistical analysis

Results are expressed as means ± SEM. Differences between two groups of unpaired data were tested using the nonparametric Mann–Whitney *U* test. Values of $p < 0.05$ were considered significant. All statistical analyses were performed using Statview software, version 5.0 (SAS Institute, Cary, NC, U.S.A.).

3. Results

3.1. Cholecalciferol postprandial response in rats

As shown in Fig. 1, the cholecalciferol response was not statistically different between refined and virgin olive oil (Fig. 1A and B). However, when the rats were force fed with olive oil supplemented in DHA, the presence of polyphenols induced a significant decrease (≈−25%) in vitamin D absorption (Fig. 1C, AUC = 12.87 ± 0.79 μmol.h/L vs 9.76 ± 0.81 μmol.h/L, $p < 0.05$). This difference remained significant after lipid adjustment (≈−43%) (Fig. 1D, AUC = 4.18 ± 0.55 μmol.h/L vs 2.37 ± 0.40 μmol.h/L, $p < 0.05$).

3.2. Vitamin D uptake efficiency by Caco-2 cells in presence of olive oil phenols

Oleuropein, hydroxytyrosol and pinoresinol were not toxic up to 500 μM. Fig. 2A shows that a mix of the 3 main olive oil polyphenols, at a total concentration of 250 μM and at ratios mimicking their respective ratios in olive oil (Table 1), significantly decreased cholecalciferol uptake from cholecalciferol-rich mixed micelles by up to 13%. When polyphenols were associated 2 by 2, all the mixes containing pinoresinol induced a significant decrease in cholecalciferol uptake (−15% and −13% for oleuropein/pinoresinol and

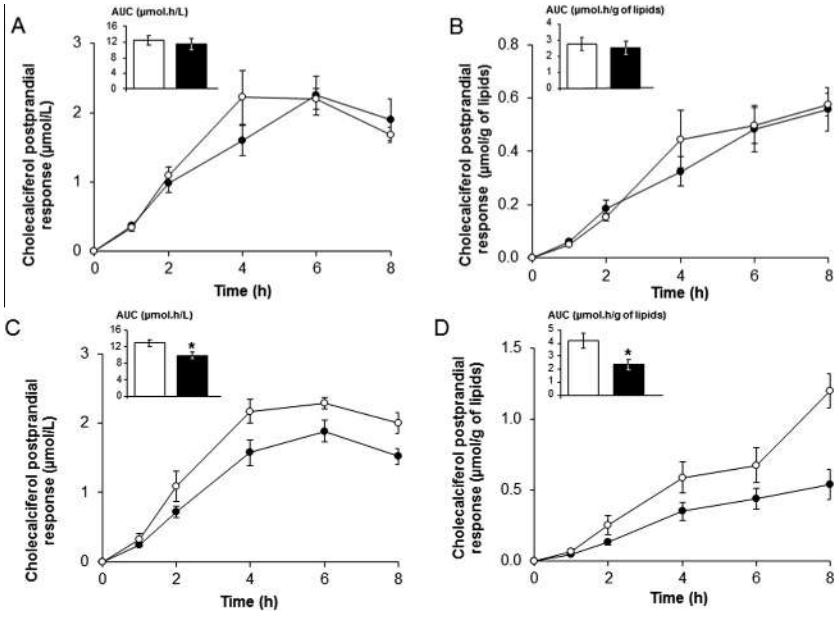

Fig. 1. Cholecalciferol postprandial response after gavage in rats. (A and B) Rats were force-fed with either a refined olive oil enriched in vitamin D (○) or with virgin olive oil enriched in vitamin D (●). (C and D) Rats were force-fed with either a refined olive oil supplemented in DHA and vitamin D (○) or with virgin olive oil supplemented in DHA and enriched in vitamin D (●). Insets: AUC, area under the postprandial curve 0–8 h. Data are means ± SEM, $n = 5$.

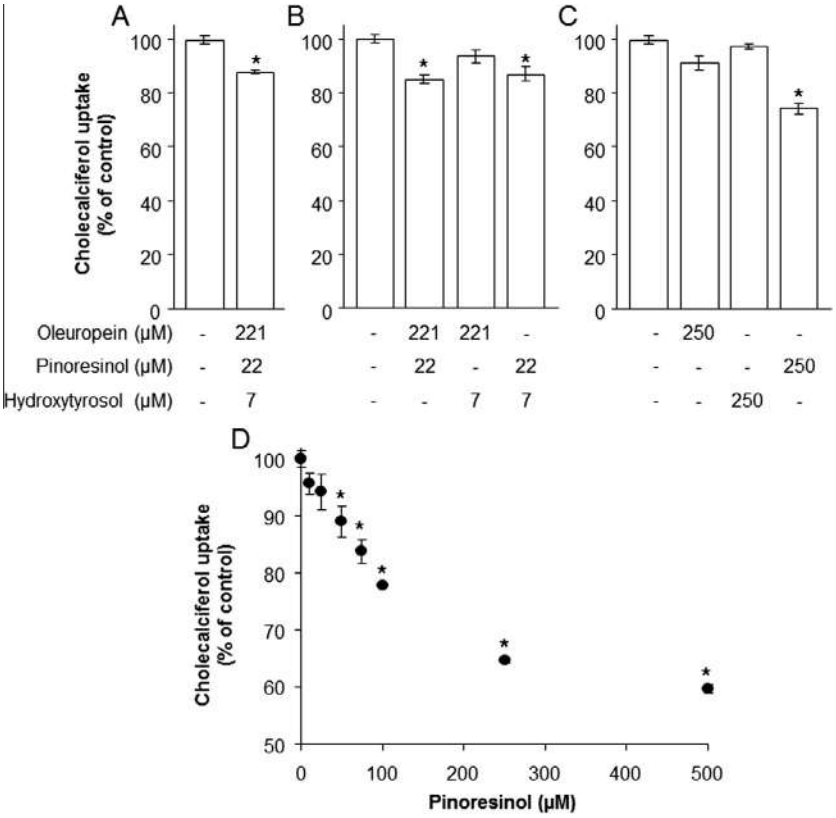

Fig. 2. Effect of phenolic compounds (oleuropein, pinoresinol and hydroxytyrosol) on vitamin D uptake by differentiated Caco-2 TC-7 monolayers. (A) Effect of a mix of 3 phenols on cholecalciferol uptake. (B) Effect of a mix of 2 phenols on cholecalciferol uptake. (C) Effect of either oleuropein, pinoresinol or hydroxytyrosol on cholecalciferol uptake. (D) Effect of increasing pinoresinol concentrations on cholecalciferol uptake. The basolateral sides received FBS-free medium. The apical sides of the cell monolayers were incubated for 60 min with vitamin D-enriched mixed micelles supplemented with different concentrations of phenols. Data are means ± SEM of 3 assays from one experiment and are representative of two independent experiments. An asterisk indicates a significant difference with the control (assay performed without phenol).

pinoresinol/hydroxytyrosol mixes, respectively, Fig. 2B). Finally, when tested alone at a concentration of 250 μM, pinoresinol also decreased vitamin D uptake by differentiated Caco-2 cells (−25%, $p < 0.05$, Fig. 2C), while the other compounds were devoid of any significant effect.

Fig. 2D shows a dose–response inhibitory effect of pinoresinol on cholecalciferol absorption by Caco-2 cells monolayers (up to −40% for 500 μM pinoresinol).

4. Discussion

It has been reported that although sunlight is clearly an important contributor to adequate vitamin D status, the exogenous sources of vitamin D found in the diet are far from negligible in many populations, especially in the winter (Cashman & Kiely, 2014; O'Mahony, Stepien, Gibney, Nugent, & Brennan, 2011). However, even with a balanced and varied diet, recommended levels may be difficult to achieve, which is why a case for supplementation or food fortification has been put forward by some authorities, especially in Northern Europe (Spiro & Buttriss, 2014). In France, it was shown that intake and vitamin D status can be improved by consumption of fortified foods such as vitamin D-fortified cow's milk, vitamin D-fortified formula or vitamin D-fortified cereal-based foods (EFSA, 2013). This is consistent with a meta-analysis of 16 randomized controlled trials worldwide in which the authors found that vitamin D-fortified foods increased vitamin D status in a dose-dependent manner (Black, Seamans, Cashman, & Kiely, 2012).

Among the vitamin D-fortified foods, milk, cereals and margarines are the most common (Spiro & Buttriss, 2014). It has been suggested that, for toxicity reasons, several fortified carriers with low concentrations are a better option for fortification polices than limited foods fortified with high levels of vitamin D (Brown, Sandmann, Ignatius, Amling, & Barvencik, 2013). In this light, another food product that could constitute a good vitamin D vector is oil. As olive oil consumption has already been associated with a decrease in cardiovascular risk factors, a protection against neurological disorders, a reduction in cancer risk (Lopez-Miranda et al., 2010), and a prevention of bone disease (Garcia-Martinez, Rivas, Ramos-Torrecillas, De Luna-Bertos, & Ruiz, 2014; Tagliaferri et al., 2014), a vitamin D-fortified olive oil could thus be a highly beneficial health product. Moreover, with the aim of producing olive oil-based health products, providing an oil rich in both vitamin D and DHA would be particularly interesting considering the fact that many people may also suffer from suboptimal health as a result of low omega 3 fatty acid intake (Salem & Eggersdorfer, 2015).

However, it has already been reported that phenolic compounds could interact negatively with fat-soluble vitamin absorption. In particular, we previously showed that the phenol naringenin could inhibit vitamin E uptake by enterocytes (Reboul et al., 2007a), which was consistent with previous data obtained in rats (Loest, Noh, & Koo, 2002). Conversely, other data suggested that the lignan sesamin could increase vitamin E absorption (Kamaleldin, Pettersson, & Appelqvist, 1995; KamalEldin et al., 2000). The question thus arises whether polyphenols from olive oil could interact with vitamin D absorption.

We first challenged this assumption in rats. Two oils supplemented in vitamin D were tested during this experiment and compared with the same refined oils. The first one was a pure virgin olive oil and the second one a virgin olive oil supplemented with DHA. No significant difference was observed between refined and

virgin olive oil regarding vitamin D absorption, whereas the presence of phenols in the olive oil containing DHA led to a significant decrease in cholecalciferol postprandial response compared to the matched control condition (refined olive oil containing DHA). This may be due to the fact that DHA was reported to decrease vitamin D absorption by itself (Goncalves et al., 2013), so the combination of both DHA and polyphenols likely induced a negative combined effect on cholecalciferol bioavailability.

In order to further understand the possible effect of polyphenols on vitamin D uptake, we then performed experiments on cultured human intestinal cells. Polyphenol concentrations used in the present study were in the dietary range, as they can reach levels up to several hundred micromoles per liter in the gastrointestinal tract (Scalbert & Williamson, 2000). We chose 3 polyphenols among the main characteristic phenolic classes of olive oil; i.e. secoiridoids (oleuropein), lignans (pinoresinol) and phenylethanoids (hydroxytyrosol). We used them at ratios representative of their respective content in our olive oil. Our results clearly highlighted a negative effect of the olive oil phenolic compounds on cholecalciferol uptake. Further investigation actually showed that this effect was only due to the presence of pinoresinol. Molecular mechanisms underlying this observation remain to be elucidated. However, it is noteworthy that the pinoresinol molecular structure is similar to a Block-Lipid Transport (BLT) structure. BLTs are small molecules that in the low nanomolar to micromolar range block selective uptake from and efflux to HDL mediated by SR-BI (Nieland, Penman, Dori, Krieger, & Kirchhausen, 2002; Nieland et al., 2004). It is therefore possible that pinoresinol can inhibit lipid transport proteins, such as SR-BI, impairing in turn cholecalciferol SR-BI-dependent uptake (Reboul et al., 2011). Further investigations are needed to fully understand the molecular mechanisms of such inhibition.

Altogether, our results show for the first time that pinoresinol can inhibit vitamin D absorption. As the pinoresinol content can highly vary between olive oils (Reboredo-Rodriguez, Gonzalez-Barreiro, Cancho-Grande, & Simal-Gandara, 2014), these results should be taken into account for enrichment of olive oil in vitamin D.

Acknowledgements

AG and CT were funded by a CIFRE grant from the ANRT [French National Association for Research and Technology] in partnership with Lesieur Company. This work was supported by both the CIFRE grant and the pOLIVd3 ANR [French National Research Agency] program ANR-10-ALIA-0008. We declare no conflicts of interest or financial interest.

References

Adams, J. S., & Hewison, M. (2010). Update in vitamin D. *Journal of Clinical Endocrinology and Metabolism, 95*(2), 471–478.

Black, L. J., Seamans, K. M., Cashman, K. D., & Kiely, M. (2012). An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *Journal of Nutrition, 142*(6), 1102–1108.

Brown, J., Sandmann, A., Ignatius, A., Amling, M., & Barvencik, F. (2013). New perspectives on vitamin D food fortification based on a modeling of 25(OH)D concentrations. *Nutrition Journal, 12*(1), 151.

Cannell, J. J., Hollis, B. W., Zasloff, M., & Heaney, R. P. (2008). Diagnosis and treatment of vitamin D deficiency. *Expert Opinion on Pharmacotherapy, 9*(1), 107–118.

Cashman, K. D., & Kiely, M. (2014). Recommended dietary intakes for vitamin D: Where do they come from, what do they achieve and how can we meet them? *Journal of Human Nutrition & Dietetics, 27*(5), 434–442.

EFSA (2013). Scientific opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. *EFSA Journal, 11*, 3408.

Garcia-Martinez, O., Rivas, A., Ramos-Torrecillas, J., De Luna-Bertos, E., & Ruiz, C. (2014). The effect of olive oil on osteoporosis prevention. *International Journal of Food Sciences and Nutrition, 65*(7), 834–840.

Goncalves, A., Gleize, B., Bott, R., Nowicki, M., Amiot, M. J., Lairon, D., ... Reboul, E. (2011). Phytosterols can impair vitamin D intestinal absorption in vitro and in mice. *Molecular Nutrition & Food Research, 55*(Suppl 2), S303–S311.

Goncalves, A., Gleize, B., Roi, S., Nowicki, M., Dhaussy, A., Huertas, A., ... Reboul, E. (2013). Fatty acids affect micellar properties and modulate vitamin D uptake and basolateral efflux in Caco-2 cells. *Journal of Nutritional Biochemistry, 24*(10), 1751–1757.

Goncalves, A., Roi, S., Nowicki, M., Dhaussy, A., Huertas, A., Amiot, M. J., & Reboul, E. (2015). Fat-soluble vitamin intestinal absorption: Absorption sites in the intestine and interactions for absorption. *Food Chemistry, 172*, 155–160.

Holick, M. F. (2007). Vitamin D deficiency. *New England Journal of Medicine, 357*(3), 266–281.

Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: A worldwide problem with health consequences. *American Journal of Clinical Nutrition, 87*(4), 1080S–1086S.

KamalEldin, A., Frank, J., Razdan, A., Tengblad, S., Basu, S., & Vessby, B. (2000). Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. *Lipids, 35*(4), 427–435.

Kamaleldin, A., Pettersson, D., & Appelqvist, L. A. (1995). Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed ad libitum. *Lipids, 30*, 499–505.

Lappe, J. M., & Binkley, N. (2015). Vitamin D and sarcopenia/falls. *Journal of Clinical Densitometry*.

Lappe, J. M., Travers-Gustafson, D., Davies, K. M., Recker, R. R., & Heaney, R. P. (2007). Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. *American Journal of Clinical Nutrition, 85*(6), 1586–1591.

Loest, H. B., Noh, S. K., & Koo, S. I. (2002). Green tea extract inhibits the lymphatic absorption of cholesterol and alpha-tocopherol in ovariectomized rats. *Journal of Nutrition, 132*(6), 1282–1288.

Lopez-Miranda, J., Perez-Jimenez, F., Ros, E., De Caterina, R., Badimon, L., Covas, M. I., ... Yiannakouris, N. (2010). Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008. *Nutrition, Metabolism and Cardiovascular Disease, 20*(4), 284–294.

Maillot, M., Vieux, F., Ferguson, E. F., Volatier, J. L., Amiot, M. J., & Darmon, N. (2009). To meet nutrient recommendations, most French adults need to expand their habitual food repertoire. *Journal of Nutrition, 139*(9), 1721–1727.

Mithal, A., Wahl, D. A., Bonjour, J. P., Burckhardt, P., Dawson-Hughes, B., Eisman, J. A., ... on behalf of the IOF Committee of Scientific Advisors (CSA) Nutrition Working Group (2009). Global vitamin D status and determinants of hypovitaminosis D. *Osteoporosis International, 20*(11), 1807–1820.

Nieland, T. J., Chroni, A., Fitzgerald, M. L., Maliga, Z., Zannis, V. I., Kirchhausen, T., & Krieger, M. (2004). Cross-inhibition of SR-BI- and ABCA1-mediated cholesterol transport by the small molecules BLT-4 and glyburide. *Journal of Lipid Research, 45*(7), 1256–1265.

Nieland, T. J., Penman, M., Dori, L., Krieger, M., & Kirchhausen, T. (2002). Discovery of chemical inhibitors of the selective transfer of lipids mediated by the HDL receptor SR-BI. *Proceedings of the National academy of Sciences of the United States of America, 99*(24), 15422–15427.

O'Mahony, L., Stepien, M., Gibney, M. J., Nugent, A. P., & Brennan, L. (2011). The potential role of vitamin D enhanced foods in improving vitamin D status. *Nutrients, 3*(12), 1023–1041.

Reboredo-Rodriguez, P., Gonzalez-Barreiro, C., Cancho-Grande, B., & Simal-Gandara, J. (2014). Quality of extra virgin olive oils produced in an emerging olive growing area in north-western Spain. *Food Chemistry, 164*, 418–426.

Reboul, E., Abou, L., Mikail, C., Ghiringhelli, O., Andre, M., Portugal, H., ... Borel, P. (2005). Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). *Biochemistry Journal, 387*(Pt 2), 455–461.

Reboul, E., Goncalves, A., Comera, C., Bott, R., Nowicki, M., Landrier, J. F., ... Borel, P. (2011). Vitamin D intestinal absorption is not a simple passive diffusion: Evidences for involvement of cholesterol transporters. *Molecular Nutrition & Food Research, 55*(5), 691–702.

Reboul, E., Klein, A., Bietrix, F., Gleize, B., Malezet-Desmoulins, C., Schneider, M., ... Borel, P. (2006). Scavenger receptor class B type I (SR-BI) is involved in vitamin E transport across the enterocyte. *Journal of Biological Chemistry, 281*(8), 4739–4745.

Reboul, E., Thap, S., Perrot, E., Amiot, M. J., Lairon, D., & Borel, P. (2007a). Effect of the main dietary antioxidants (carotenoids, gamma-tocopherol, polyphenols, and vitamin C) on alpha-tocopherol absorption. *European Journal of Clinical Nutrition*.

Reboul, E., Thap, S., Tourniaire, F., Andre, M., Juhel, C., Morange, S., ... Borel, P. (2007b). Differential effect of dietary antioxidant classes (carotenoids, polyphenols, vitamins C and E) on lutein absorption. *British Journal of Nutrition, 97*(3), 440–446.

Salem, N., Jr., & Eggersdorfer, M. (2015). Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? *Current Opinion in Clinical Nutrition and Metabolic Care, 18*(2), 147–154.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition, 130*(8S Suppl), 2073S–2085S.

Spiro, A., & Buttriss, J. L. (2014). Vitamin D: An overview of vitamin D status and intake in Europe. *Nutrition Bulletin, 39*(4), 322–350.

Tagliaferri, C., Davicco, M. J., Lebecque, P., George, S., Amiot, M. J., Mercier, S., ... Coxam, V. (2014). Olive oil and vitamin D synergistically prevent bone loss in mice. *PLoS ONE, 9*(12), e115817.